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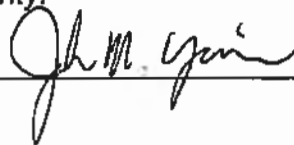
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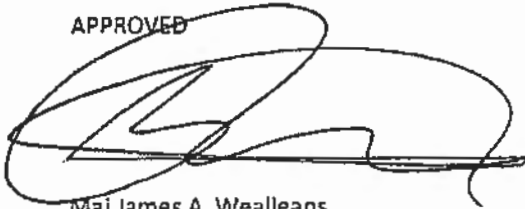
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The Effect of 8.25% Sodium Hypochlorite on Dental Pulp Dissolution and Dentin Flexural Strength and Modulus

Maj James K.T. Cullen

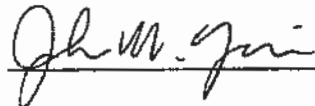
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30 JUN 2015

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Abstract

Introduction: The purpose of this study was to evaluate the effect of various concentrations of sodium hypochlorite (NaOCl), including 8.25%, on dental pulp dissolution and dentin flexural strength and modulus. **Methods:** 60 dental pulp samples and 55 plane-parallel dentin bars were retrieved from extracted human teeth. Five test groups (n=10) were formed consisting of a pulp sample and dentin bar immersed in various NaOCl solutions. The negative control group (n=5) consisted of pulp samples and dentin bars immersed in saline. The positive control group (n=5) consisted of pulp samples immersed in 8.25% NaOCl without a dentin bar. Every six minutes for one hour, the solutions were refreshed. The dentin bars were tested for flexural strength and modulus with a three-point bend test. The time until total pulp dissolution and any changes in dentin bar flexural strength and modulus for the different NaOCl solutions were statistically analyzed. **Results:** An increase in NaOCl concentration showed a highly significant decrease in pulp dissolution time. The pulp dissolution property of 8.25% NaOCl was significantly faster than any other tested concentration of NaOCl. The presence of dentin did not have a significant effect on the dissolution capacity of NaOCl if the solutions were refreshed. NaOCl concentration did not have a statistically significant effect on dentin flexural strength or modulus.

Conclusions: Dilution of NaOCl decreases its pulp dissolution capacity. Refreshing the solution is essential to counteract the effects of dentin. In this study, NaOCl did not have a significant effect on dentin flexural strength or modulus.

Key Words: dentin, dental pulp, dissolution, flexural modulus, flexural strength, sodium hypochlorite

Introduction

The main goal of endodontic treatment is the prevention or treatment of apical periodontitis (1) of which bacteria play a critical role (2). Thorough debridement of pulp tissue and disinfection of the canal space is essential in achieving this goal (3). Instrumentation alone is not able to fully debride the canal space, especially in irregularly shaped canals and isthmuses (4), therefore an effective disinfection agent is required for successful endodontic treatment.

In 1915, the English chemist Henry Dakin recommended using a “weak neutral solution of sodium hypochlorite” (0.5 - 0.6%) to irrigate infected wounds (5). As early as 1920, sodium hypochlorite (NaOCl) became a main irrigant in endodontics (6) and is currently the preferred endodontic irrigant. According to Zehnder, the ideal root canal irrigant should: 1) have a broad antimicrobial spectrum, 2) dissolve necrotic pulp tissue, 3) inactivate endotoxin, 4) prevent or remove the smear layer, and 5) be nontoxic and noncaustic to periodontal tissues with little potential for anaphylactic reactions. Although NaOCl does not fully meet his description of an ideal irrigant, it meets many of his requirements (3). Besides having antibacterial properties, it is readily available, inexpensive, and has a long shelf life (7). NaOCl’s ability to dissolve dental pulp tissue is beneficial during endodontic therapy.

It is common for dentists to use ordinary household bleach purchased from local stores as their source for the sodium hypochlorite used during endodontic irrigation (8). Since there may be many different concentrations of sodium hypochlorite available, the dentist should be aware of the concentration they are using and any possible effects on treatment. Historically,

concentrations of 5.25% and 6% NaOCl were available and, in many cases, companies have discontinued their production. In 2012, a concentrated 8.25% NaOCl became available, however many dentists are not aware of this change. These solutions are frequently used “full strength” or they may be diluted with water to a concentration as low as 0.5% (9). The effect of 8.25% NaOCl on pulp dissolution and on the physical properties of dentin has not been previously evaluated.

The purpose of this study was to evaluate the effect of various concentrations of NaOCl, including 8.25%, on dental pulp dissolution and the flexural strength and modulus of dentin and to determine if regularly refreshing the NaOCl could overcome the negative effect dentin has on pulp dissolution. The null hypothesis was that an increase in NaOCl concentration would not have a significant impact on pulp dissolution or dentin flexural strength and modulus.

Materials and Methods

Freshly extracted permanent mandibular molars were stored in 0.5% chloramine-T. The teeth were sectioned with bolt cutters in a facial-lingual direction (Fig. 1A) and the pulps were removed (Fig. 1B) and rinsed with physiologic saline to remove any debris and blotted dry. Samples were collected from the pulp with the use of 2mm diameter biopsy punches (Uni-Punch, Premier, Plymouth Meeting, PA) (Fig. 1C/D), then weighed to the nearest 10^{-4} grams (HR-200, Orion Analytical Balance Series, A&D Weighing, Milpitas, CA). This standardized the samples both by weight and surface area. Five experimental groups containing 10 samples each were

tested. Both a negative and positive control group containing 5 samples each were also tested. All samples were stored in physiologic saline at 4°C until tested.

Standardized plane-parallel dentin bars were produced by longitudinally sectioning the teeth into 1 mm x 1 mm x 10 mm segments with an Isomet low-speed (LS) saw (Buehler, Lake Bluff, IL) equipped with a 0.30 mm thick, Series 15, high concentration wafering diamond blade (Buehler, Lake Bluff, IL) (Fig. 1E/F). No enamel or cementum were included in the dentin bars. These samples were also stored in physiologic saline until tested.

Test samples were randomly assigned to a group according to NaOCl concentration: 0.5%, 2%, 4.125%, 6.0%, and 8.25%. The reduced concentration NaOCl solutions were prepared by diluting 8.25% NaOCl (Clorox Concentrated Bleach, Oakland, CA) with distilled water. There was a positive control group for the pulp samples and negative control groups for both pulp samples and dentin bars.

Immediately prior to testing, a pulp sample and dentin bar were blotted dry and placed into a 74 µm pore size Netwell™ insert (Corning Life Sciences, Tewksbury, MA) (Fig. 1G) which was placed into one well of a multi-well plate (Fig. 1H). Each well received 2 mL of one of the NaOCl solutions, applied over the dentin bar first, to immerse both the pulp and dentin bar in the solution. The positive control group only included a pulp sample, without a dentin bar, and used 8.25% NaOCl. Physiologic saline was used for the negative control group.

Every 6 minutes, the Netwell™ insert, including the pulp sample and dentin bar, were removed from the well along with the remaining solution. Then the Netwell™ insert was placed back into the well and 2 mL of a fresh solution of the same concentration of NaOCl was added by the same method. The time until complete dissolution of the pulp tissue was measured to the nearest second. In order to standardize the times that all dentin bars were immersed in NaOCl, the solutions were replaced in all wells every 6 minutes for a total of 60 minutes. At that time, any remaining pulp samples, including those from the negative control group, were removed from solution, blotted dry, and weighed. The initial and final weights of the samples were compared. The time required until total pulp dissolution of the test groups was statistically analyzed while the time for dissolution of the positive control group was compared to the test group with 8.25% NaOCl to determine whether or not the presence of dentin had a significant effect.

This study also measured the effect of the different NaOCl concentrations on the flexural strength and modulus of the dentin bars. After 60 minutes immersion, the dentin bars were removed from the solutions, rinsed with saline and their width and depth were measured with an electronic digital caliper (GA182, Grobet Vigor, Carlstadt, NJ). Using a universal testing machine (Instron, Norwood, MA), each specimen was placed on a three-point bending test device and a central load was applied with a head diameter of 2 mm at a crosshead speed of 0.25 mm/min. The flexural strength was calculated using the equation:

$$\sigma_{FS} = \frac{3Fl}{2bd^2}$$

where F is the loading force at the fracture point, l is the length of the support span, b is the width, and d is the depth of the specimen. The mean and standard deviation were calculated.

Flexural modulus was determined from the slope of the linear region of the load-deflection curve using the analytical software (Bluehill 2, version 2.31, Instron, Norwood, MA).

Statistical Analysis: Means, standard deviations, medians and inter-quartile range (IQR) were calculated for measured interval variables. Differences of interval variables among concentrations were tested with ANOVA. In the event the null hypothesis was rejected, Tukey contrasts were used to compare means. In the event the null hypothesis was accepted, post-hoc power analyses were performed. A paired t-test was employed to compare pre- and post-experiment means in the pulp negative control.

Results

All pulp tissue samples were completely dissolved within the 1 hour test time, except for the negative control, which had no significant change in weight. There were highly significant differences ($P = .00001$) between all groups, except between the 8.25% NaOCl group and the positive control group in which there was no significant difference (Fig. 2). The concentration of NaOCl did not have a statistically significant effect on dentin flexural strength (Fig. 3A) or modulus (Fig. 3B).

Discussion

Many dentists use NaOCl as an endodontic irrigant due to its antibacterial properties and ability to dissolve pulp tissue (7). NaOCl is a strong base and derives its ability to dissolve

tissues from its high pH (>11). The pH decreases when exposed to amino acids by releasing hydroxyl ions, forming water and salt. NaOCl solution also contains hypochlorous acid that acts as a solvent when in contact with organic tissues. Hydroxyl ions and hypochlorous acid lead to amino acid degradation and hydrolysis (10). NaOCl's antibacterial effects are due to the hypochlorous acid, which contains active chlorine, a strong oxidizing agent. The chlorine irreversibly oxidizes essential enzymes of the bacteria, disrupting its metabolic functions. Chlorine may interact with cytoplasmic components, creating toxic compounds that can destroy the bacteria (11). NaOCl has been shown to have a greater dissolution effect on necrotic tissues than normal, healthy tissue (12). Unfortunately, it has a poor ability to inactivate endotoxin (13) and does not remove or prevent formation of the smear layer (14).

There have been multiple case reports on NaOCl incidents that include clinical symptoms of severe pain, edema, bleeding, ecchymosis, and potential for infection (15-18). As a result, many have cautioned against the use of a concentrated NaOCl solution due to its cytotoxicity and potential for clinical complications if extruded into the periradicular tissues (9, 17-19). They recommend a diluted concentration that still retains its disinfective properties (9,18). Concentrations as low as 0.5% and 1% NaOCl have been recommended (9) and studies by Rocas et al and Siqueira et al have shown that solutions of 1% and 2.5% NaOCl still maintain their antimicrobial effect (11, 20).

Alternatively, studies by Harrison et al on the clinical toxicity of irrigants found that the use of 5.25% NaOCl did not result in an increase in interappointment pain (21, 22). Another

study showed that the dilution of 5.25% NaOCl resulted in a significant decrease in the ability to dissolve necrotic tissue (23).

Many studies have shown that the tissue dissolution capabilities of NaOCl can increase with an increase in concentration (24-26), however they used dermal connective tissues from rats and bovine muscle. Few studies have been completed on human dental pulp tissue, which may be more clinically relevant. In a study that did use human dental pulp, only a concentration of 2.5% NaOCl was investigated (27). That study indicated that the presence of dentin would have a negative effect on the pulp dissolution capability of NaOCl, but the authors did not refresh the solution, as would be done in a clinical situation.

This study showed no significant difference in pulp dissolution time between the 8.25% NaOCl solution with the dentin bar and the positive control without the dentin bar. Since the solutions were refreshed, the presence of dentin did not have a significant effect on the dissolution capacity of NaOCl. This emphasizes the importance of consistently refreshing the NaOCl solution during treatment to minimize the negative effects of dentin on the pulp dissolution properties of NaOCl.

Other research has looked at NaOCl's effect on dentin, and shown that an increase in NaOCl concentration will have a negative impact on the flexural strength of dentin (28-30). While an increase in NaOCl concentration can increase its penetration into dentinal tubules, potentially creating a greater antimicrobial effect (31), it may also cause a greater increase in decalcification of the root canal dentin (32). While decalcification has an impact on the inorganic

portion of dentin, NaOCl also has an effect on the organic portion, which constitutes approximately 20% of dentin by weight. Most of this consists of type I collagen, which contributes to the mechanical properties of dentin (33).

Three main factors make endodontically treated teeth more susceptible to fracture: loss of tooth structure, altered proprioception, and altered physical properties of dentin (28). NaOCl may have an impact on these physical properties and play a role in cervical fractures which could ultimately lead to failure (30). However, the significance of NaOCl's effect on dentin is unknown and this study found no significant difference between the flexural strength and modulus of dentin in the control group and any of the test groups. This finding counters other studies claiming that NaOCl has a negative effect on dentin flexural strength and modulus (28-30). This result may have occurred because there was no way to account for many factors that could have affected the dentin flexural strength and modulus prior to testing, such as the age of the patient, any systemic conditions, or normal variability between individuals.

In conclusion, the null hypothesis for pulp dissolution was rejected, but it was accepted in regards to dentin flexural strength and modulus. This study found that: 1) there is an increase in pulp dissolution capability of NaOCl with an increase in concentration, 2) that the tissue dissolution property is not negated by dentin if the NaOCl is refreshed, and 3) that there is no significant decrease in dentin flexural strength or modulus with an increase in NaOCl concentration. It is important for dental providers to be aware of the concentration of NaOCl they are using during endodontic treatment, and using full-strength (8.25%) NaOCl will quickly and

effectively dissolve pulp tissue with little effect on dentin's physical properties. More research is necessary to discover a solution that satisfies all of the criteria for an ideal endodontic irrigant.

Figure Legends



Figure 1. Specimen preparation and testing. (A) Tooth sectioned; (B) Pulp removed; (C,D) Pulp sample collected with biopsy punch; (E,F) Dentin bar fabrication; (G) Netwell™ inserts in multi-well plate; (H) Pulp sample and dentin bar in solution.

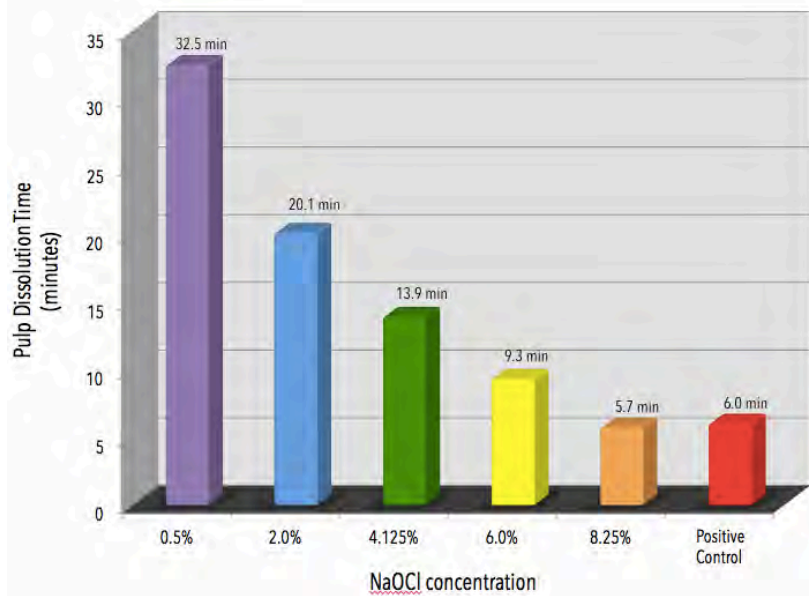


Figure 2. The mean times for total pulp dissolution in various concentrations of NaOCl. There were highly significant differences ($P = .00001$) between all groups, except between the 8.25% NaOCl group and the positive control group.

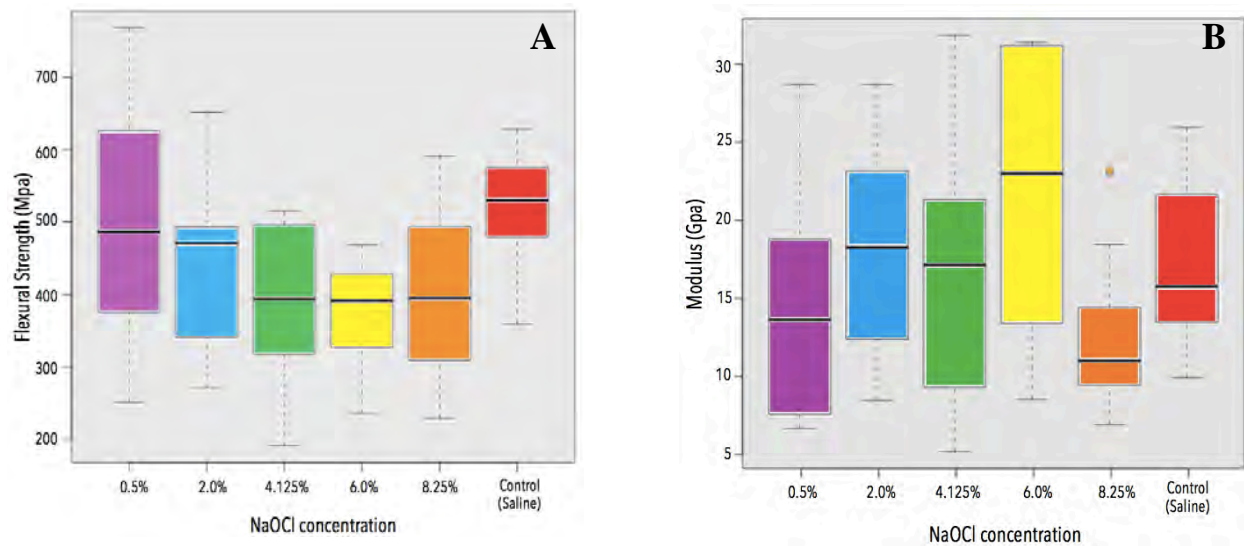


Figure 3. Box plots of flexural strength (A) and flexural modulus (B) for various concentrations of NaOCl. Bold line shows median. NaOCl concentration had no statistically significant effect on dentin flexural strength or modulus.

References

1. Trope M. The vital tooth – its importance in the study and practice of Endodontics. *Endod Topics* 2003;5:1.
2. Kakehashi S, Stanley HR, Fitzgerald RJ. The effects of surgical exposure of dental pulps in germ-free and conventional laboratory rats. *Oral Surg Oral Med Oral Pathol* 1965;18:340-8.
3. Zehnder M. Root canal irrigants. *J Endod* 2006;32:389-98.
4. Byström A, Sundqvist G. Bacteriologic evaluation of the efficacy of mechanical root canal instrumentation in endodontic therapy. *Scand J Dent Res* 1981;89:321-8.
5. Dakin HD. On the use of certain antiseptic substances in treatment of infected wounds. *Br Med J* 1915;2:318 –20.
6. Crane AB. A predictable root canal technique. Philadelphia: Lea & Febiger, 1920.
7. Frai S, Ng YL, Gulabivala K. Some factors affecting the concentration of available chlorine in commercial sources of sodium hypochlorite. *Int Endod J* 2001;34:206-15.
8. Jungbluth H, Peters C, Peters O, Sener B, Zehnder M. Physicochemical and pulp tissue dissolution properties of some household bleach brands compared with a dental sodium hypochlorite solution. *J Endod* 2012;38:372-5.
9. Spangberg L, Engström B, Langeland K. Biologic effects of dental materials. 3. Toxicity and antimicrobial effect of endodontic antiseptics in vitro. *Oral Surg Oral Med Oral Pathol* 1973;36:856-71.
10. Estrela C, Estrela CRA, Barbin E, Spano J, Marchesan M, Pecora J. Mechanism of action of sodium hypochlorite. *Braz Dent J* 2002;13:113-7.

11. Rocas I, Siqueira J. Comparison of the in vivo antimicrobial effectiveness of sodium hypochlorite and chlorhexidine used as root canal irrigants: a molecular microbiology study. *J Endod* 2011;37:143-50.
12. Austin JH, Taylor HD. Behavior of hypochlorite and of chloramine-T solutions in contact with necrotic and normal tissue in vivo. *J Exp Med* 1918;27:627-33.
13. Buck R, Eleazer P. Detoxification of endotoxin by endodontic irrigants and calcium hydroxide. *J Endod* 2001;27:325-7.
14. Uroz-Torres, D. Effectiveness of the EndoActivator System in removing the smear layer after root canal instrumentation. *J Endod* 2010;36:308-11.
15. Mehra P, Clancy C, Wu J. Formation of facial hematoma during endodontic therapy. *J Am Dent Assoc* 2000;131:67-71.
16. Chaudhry H, Wildan TM, Popat S, Anand R, Dhariwal D. Before you reach for bleach... *Br Dent J* 2011;210:157-60.
17. Gernhardt CR, Eppendorf K, Kozlowski A, Brandt M. Toxicity of concentrated sodium hypochlorite used as an endodontic irrigant. *Int Endod J* 2004;37:272-80.
18. Hulsmann M, Hahn W. Complications during root canal irrigation: literature review and case reports. *Int Endod J* 2000;33:186-93.
19. Clarkson RM, Moule AJ. Sodium hypochlorite and its use as an endodontic irrigant. *Aust Dent J* 1998;43:250-6.
20. Siqueira J, Rocas I, Favieri A, Lima K. Chemomechanical reduction of the bacterial population in the root canal after instrumentation and irrigation with 1%, 2.5%, and 5.25% sodium hypochlorite. *J Endod* 2000;26:331-4.

21. Harrison J, Svec T, Baumgartner J. Analysis of clinical toxicity of endodontic irrigants. *J Endod* 1978;4:6-11.
22. Harrison J, Baumgartner J, Zielke D. Analysis of interappointment pain associated with the combined use of endodontic irrigants and medicaments. *J Endod* 1981;7:272-6.
23. Hand R, Smith M, Harrison J. Analysis of the effect of dilution on the necrotic tissue dissolution property of sodium hypochlorite. *J Endod* 1978;4:60-4.
24. Abou-Rass M, Oglesby SW. The effects of temperature, concentration, and tissue type on the solvent ability of sodium hypochlorite. *J Endod* 1981;7:376 –7.
25. Moorer WR, Wesselink PR. Factors promoting the tissue dissolving capability of sodium hypochlorite. *Int Endod J* 1982; 15:87–96.
26. Stojicic S, Zivkovic S, Qian W, Zhang H, Haapasalo M. Tissue dissolution by sodium hypochlorite: effect of concentration, temperature, agitation, and surfactant. *J Endod* 2012;36:1558-62.
27. Slutzky-Goldberg I, Hanut A, Matalon S, Baev V, Slutzky H. The effect of dentin on the pulp tissue dissolution capacity of sodium hypochlorite and calcium hydroxide. *J Endod* 2013;39:980-3.
28. Sim T, Knowles J, Ng Y, Shelton J, Gulabivala K. Effect of sodium hypochlorite on mechanical properties of dentine and tooth surface strain. *Int Endod J* 2001;34:120-32.
29. Marending M, Luder H, Brunner T, Knecht S, Stark W, Zehnder M. Effect of sodium hypochlorite on human root dentine – mechanical, chemical and structural evaluation. *Int Endod J* 2007;40:786–93.

30. Grigoratos D, Knowles J, Ng Y-L, Gulabivala K. Effect of exposing dentine to sodium hypochlorite and calcium hydroxide on its flexural strength and elastic modulus. *Int Endod J* 2001;34:113-9.
31. Zou L, Shen Y, Li W, Haapasalo M. Penetration of sodium hypochlorite into dentin. *J Endod* 2010;36:793-6.
32. Sayin TC, Cehreli ZC, Deniz D, Akcay A, Tuncel B, Dagli F, Gozukara H. Time-dependent decalcifying effects of endodontic irrigants with antibacterial properties. *J Endod* 2009;35:280-3.
33. Luukko K, Kettunen P, Fristad I, Berggreen E. Structure and functions of the dentin-pulp complex. In: Cohen's *Pathways of the Pulp*, 10th ed, 2011; St. Louis, MO, USA, Mosby-Elsevier: Ch 12:452-503.